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Previews

Epithelial-Mesenchymal Transition and the Stem Cell Phenotype

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Epithelial-mesenchymal transition (EMT) is a developmental process in which epithelial cells acquire the motile, migratory properties of mesenchymal cells. In a recent issue of *Cell*, Mani et al. (2008) show that induction of EMT stimulates cultured breast cells to adopt characteristics of stem cells.

Epithelial-mesenchymal transition (EMT) is a physiological phenotypic shift in which epithelial cells break down cell-cell and cell-extracellular matrix connections and then migrate to other locations in the body. Once the migrating mesenchymal cells have reached their destination, they can undergo a reverse EMT, a mesenchymal-epithelial transition (MET). During embryonic development, EMT creates cells that act as progenitors of many different tissues. For example, gastrulation EMT produces the mesoderm, giving rise to a wide range of cell types, including muscle, bone, and connective tissues, whereas neural crest delamination EMT gives rise to glial and neuronal cells, adrenal glandular tissues, pigment-containing cells of the epidermis, and skeletal and connective tissues (Figures 1A and 1B) (Shook and Keller, 2003). EMT functions in adults to facilitate organ morphogenesis as well as tissue regeneration and regrowth during wound repair. However, there is an increasing awareness that EMT-associated processes can contribute to invasive and metastatic tumor growth (Figure 1C); consequently, considerable effort is being expended to identify the mechanisms involved in EMT induction and maintenance (Hugo et al., 2007; Thiery and Sleeman, 2006). EMT can be induced in mammary epithelial cells by exposure to certain cytokines, such as TGF-β1, or by exogenous expression of various transcription factors, including those of the Twist or Snail families (Thiery and Sleeman, 2006; Zavadil and Bottinger, 2005). Significantly, activation of these processes in breast cancer cells greatly increases their invasive and metastatic potential, but some argument still exists as to exactly how EMT participates in progression of tumors to metastasis (Hugo et al., 2007).

How an induced EMT might impact the various epithelial cells in the mammary gland is unknown. A developmental hierarchy has been demonstrated in this tissue in which stem cells give rise to both the outer myoepithelial layer and the inner layer of luminal epithelial cells, which define the bilayered architecture of the branching mammary gland (Shackleton et al., 2006; Stingl et al., 2006; Villadsen et al., 2007). Normal human mammary tissue contains a subpopulation of $EpCam^{high}/CD49f^{high}$ stem cells that give rise to mammospheres and to bilayered, branching structures in 3D culture (Villadsen et al., 2007). A direct etiological link between normal stem cells and cancer has been postulated due to the observation that cells within tumors exhibit attributes of stem cells, but the exact relationship remains elusive. Al Hajj Al-Hajj et al. (2003) isolated a $CD44^{high}/CD24^{low}$ subpopulation of breast cancer pleural effusions and showed that these cells generated tumors in a xenograft model more effectively than did the majority population of $CD44^{low}/CD24^{high}$ cells. The $CD44^{high}/CD24^{low}$ cells were subsequently designated as "cancer stem cells" (CSCs), and this discovery has fueled investigation of CSCs as the metastatic component of cancer (Hermann et al., 2007). In the present work, Mani et al. (2008) demonstrate that cells from normal human and mouse mammary tissues with functional properties of stem cells are also $CD44^{high}/CD24^{low}$ and, enticingly, that the surface phenotype can be induced by EMT (Figure 1D). Thus, the authors have identified a potential link between EMT, which endows cells with migratory and invasive capabilities, and cells with properties of CSC.

Mani et al. (2008) found that cultured normal human mammary epithelial cells contained both $CD44^{high}/CD24^{low}$ and $CD44^{low}/CD24^{high}$ subpopulations and that isolated $CD44^{high}/CD24^{low}$ cells displayed a mesenchymal morphology and expressed characteristic mesenchymal markers. This population also demonstrated many of the properties of stem cells, including enhanced mammosphere-forming ability and differentiation into cells expressing lineage-specific markers of myoepithelial or luminal epithelial cells. The isolated $CD44^{high}/CD24^{low}$ cells, when grown on tissue culture plastic, regenerated both $CD44^{high}/CD24^{low}$ and $CD44^{low}/CD24^{high}$ cell populations, whereas culture of $CD44^{low}/CD24^{high}$ cells did not give rise to $CD44^{high}/CD24^{low}$ cells. Analysis of the stem cell population from mouse mammary glands ($CD49f^{high}/CD24^{medium}$) showed that cells that could regenerate mammary glandular structures when injected into cleared fat pads also displayed a mesenchymal morphology and expressed mesenchymal markers. These experiments suggest either that stem cells normally have characteristics associated with mesenchymal cells or that a subpopulation of normal mammary epithelial cells possesses both stem cell-like properties and the mesenchymal phenotype.

In a pivotal series of experiments, Mani and colleagues found that normal mammary epithelial cells could be induced to adopt the $CD44^{high}/CD24^{low}$ expression profile when exposed to TGF-β1 for 12 days or when EMT-inducing transcription factors Snail or Twist were conditionally overexpressed for the same time period.

The resulting population displayed mesenchymal morphology and surface markers, and it grew much more effectively in mammosphere culture. These experiments suggested that EMT was sufficient to induce a population with characteristics of stem cells, and they pointed to the exciting possibility that the processes by which developmental EMT generates numerous cell types from the mesoderm or neural crest may be related to the processes by which breast progenitor cells give rise to cells of the mammary gland. One important unresolved question is how treatment with TGF-β1 or expression of Snail or Twist leads to the evolution of the $CD44^{high}/CD24^{low}$ population. One possibility is that existing $CD44^{low}/CD24^{high}$ cells were converted to $CD44^{high}/CD24^{low}$ cells by the EMT-inducing stimulus; this hypothesis suggests the remarkable possibility that the majority $CD44^{low}/CD24^{high}$ subpopulation has the potential to adopt stem cell-like characteristics. An alternative possibility is that the EMT stimuli inhibited proliferation of the $CD44^{low}/CD24^{high}$ subpopulation and also either induced proliferation or inhibited differentiation of the $CD44^{high}/CD24^{low}$ cells. This model implies a potential role for EMT-associated processes in normal stem cell function, either their expansion or maintenance. Defining the response of isolated $CD44^{low}/CD24^{high}$ cells to the EMT stimuli could potentially resolve this issue.

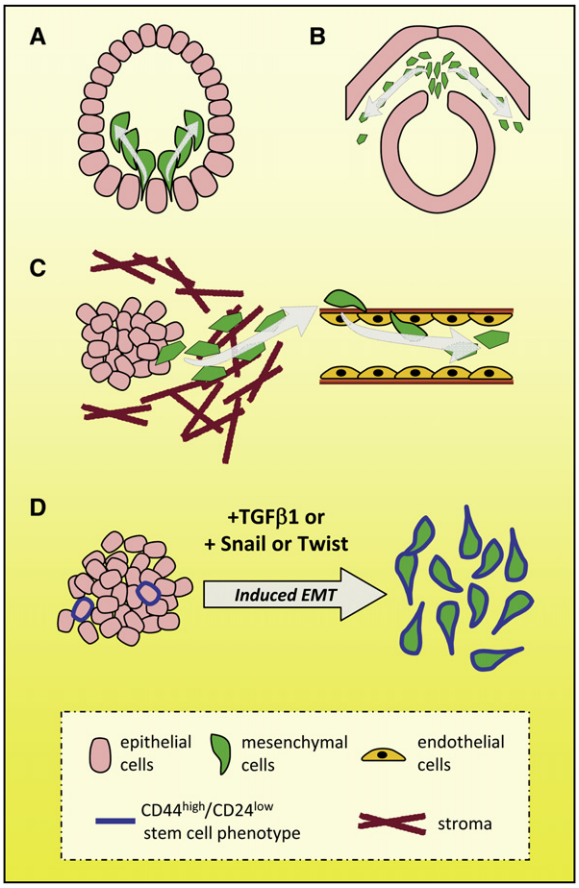

Figure 1. EMT Is a Developmental Process that Can Be Hijacked during Pathogenesis

(A) Gastrulation. Formation of the mesoderm by EMT produces the third embryonic layer.

(B) Neural crest delamination. EMT of neuroepithelial cells is the key step for induction of the neural crest.

(C) Cancer progression. EMT facilitates intravasation of tumor cells into blood or lymph vessels and subsequent formation of distant metastases.

(D) In the new report by Mani et al. (2008) in *Cell*, experimentally induced EMT in mammary epithelial cells is shown to generate cells with a $CD44^{high}/CD24^{low}$ phenotype: a phenotype that gained notoriety as a putative marker of breast cancer stem cells.

An exciting implication of these results is that there may be a direct relationship between EMT and the phenomenon of CSCs. As discussed above, breast CSCs were defined as a $CD44^{high}/CD24^{low}$ subpopulation of cancer cells that is enriched for tumorigenic cells (Al-Hajj et al., 2003); Mani et al. (2008) now show that EMT inducers confer upon breast cancer cells both increased malignancy and the $CD44^{high}/CD24^{low}$ expression pattern. Because a number of studies have shown that inducers of EMT can cause cancer cells to become more tumorigenic (Hugo et al., 2007), it may be that CSCs are not distinct entities but rather tumor cells that transiently acquire stem cell-like properties as a consequence of a regulatable EMT. This model may provide insight into current questions about the specific role of EMT in tumor progression (Hugo et al., 2007). Most tumors do not harbor a significant proportion of cells with traditional characteristics of EMT, such as increased expression of the intermediate filament protein vimentin or the acquisition of mesenchymal morphology (Hugo et al., 2007); identification of $CD44^{high}/CD24^{low}$ as specifically relevant characteristics of EMT-facilitated tumor malignancy could help to resolve this long-standing and critical issue.

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